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Corresponding Author: Dr. Amparo Lopez-Rubio,

Corresponding Author's Institution:

First Author: Rocio Perez-Masia

Order of Authors: Rocio Perez-Masia; Ruben Lopez-Nicolas; Maria Jesús Periago; Gaspar Ros; Jose Maria Lagaron; Amparo Lopez-Rubio

Abstract: In this work, two different technologies (electrospraying and nanospray drying) were evaluated for encapsulation of folic acid using a whey protein concentrate (WPC) matrix and a commercial resistant starch. The morphology of the capsules, molecular organization of the matrices, encapsulation efficiency, and stability of folic acid within the capsules under different storage conditions and upon thermal exposure were studied. Results showed that spherical submicro- and microcapsules were obtained through both techniques, although electrospraying led to smaller capsule sizes and to an enhanced control over their size distribution. Greater encapsulation efficiency was observed using WPC as encapsulating matrix, probably related to interactions between the protein and folic acid which favoured the incorporation of the bioactive. The best results in terms of bioactive stabilization in the different conditions assayed were also obtained for the WPC capsules, although both materials and encapsulation techniques led to improved folic acid stability, especially under dry conditions.

Highlights

- Folic acid was encapsulated through nanospray drying and electrospraying
- A whey protein and a resistant starch were used as encapsulating matrices
- Spherical nano-, submicro- and microcapsules were obtained through both techniques
- Greater encapsulation efficiency was observed for the protein-based capsules
- Both materials and encapsulation techniques led to improved folic acid stability

Encapsulation of folic acid in food hydrocolloids through nanospray drying and electrospraying for nutraceutical applications

Rocío Pérez-Masiá[1], Rubén López-Nicolás[2], Maria Jesús Periago[2], Gaspar Ros[2], Jose M. Lagaron[1], Amparo López-Rubio[1]*

[1]Novel Materials and Nanotechnology Group, Institute of Agrochemistry and Food Technology (IATA-CSIC), Avda. Agustin Escardino 7, 46980 Paterna (Valencia), Spain

[2]Department of Food Technology, Food Science and Nutrition, Faculty of Veterinary Sciences, Regional Campus of International Excellence "Campus Mare Nostrum". University of Murcia, Espinardo 30071 (Murcia), Spain.

*Corresponding author: Tel.: +34 963900022; fax: +34 963636301.

E-mail address: amparo.lopez@iata.csic.es (A. Lopez-Rubio)

Abbreviated running title: "Encapsulation of folic acid through nanospray drying and electrospraying"

Abstract

In this work, two different technologies (electrospraying and nanospray drying) were evaluated for the encapsulation of folic acid using both a whey protein concentrate (WPC) matrix and a commercial resistant starch. The morphology of the capsules, molecular organization of the matrices upon encapsulation, encapsulation efficiency, and stability of the folic acid within the capsules under different storage conditions and upon thermal exposure were studied. Results showed that spherical nano-, submicro- and microcapsules were obtained through both techniques, although electrospraying led to smaller capsule sizes and to an enhanced control over their size distribution. Greater encapsulation efficiency was observed using WPC as encapsulating matrix, probably related to interactions between the protein and folic acid which favoured the incorporation of the bioactive. The best results in terms of bioactive stabilization in the different conditions assayed were also obtained for the WPC capsules, although both materials and encapsulation techniques led to improved folic acid stability, especially under dry conditions.

Keywords: encapsulation, electrospraying, spray drying, food hydrocolloids, folic acid

1. Introduction

The encapsulation of nutraceutical and functional ingredients is an area of increased interest over the last years which seek to protect these products from adverse environmental conditions and, thus, increase their shelf-life and assure their health-promoting properties. Moreover, the encapsulation of these components also enables their incorporation into different food matrices which results in novel functional food products with potential health benefits.

A variety of techniques have been used to encapsulate functional components, such as nanoemulsions (Silva, Cerqueira & Vicente, 2012), coacervation (de Conto, Grosso & Gonçalves, 2013; Tamjidi, Nasirpour & Shahedi, 2012), extrusion methods (Li, Chen, Sun, Park & Cha, 2011), fluidized bed coating (Zuidam & Simoni, 2010), spray cooling (Gibbs, Selm, Catherine & Mulligan, 1999) or spray drying (Murugesan & Orsat, 2012). Among these methods, spray drying is nowadays the most common and cheapest technology in the food industry to produce microencapsulated additives for food applications (Gharsallaoui, Roudaut, Chambin, Voilley & Saurel, 2007). The active material to be encapsulated through the spray drying technique is dispersed in a carrier polymer solution which is atomized into small droplets. The solvent is evaporated using a warmed gas and the resulting solid capsules are collected as dry powder (Gibbs et al., 1999; Gharsallaoui et al., 2007). It is worth noting that this technology can be used with aqueous solutions, thus avoiding the use of organic solvents which could generate toxicity problems in contact with food. Nevertheless, it needs relatively high temperatures to eliminate the water from the polymeric/biopolymeric solutions, fact that could affect the stability of the bioactive ingredient. Apart from these well-known encapsulation techniques, electrospinning has recently arisen as an alternative

technology that can also be used for encapsulation (Torres-Giner, Martínez-Abad, Ocio & Lagaron, 2009; Lopez-Rubio & Lagaron, 2012). Besides being a very simple technique, some advantages of electrospinning for encapsulation include that neither temperature nor organic solvents are needed, thus, being an ideal method for protecting sensitive encapsulated ingredients. Electrospinning makes use of high voltage electric fields to produce electrically charged jets from viscoelastic polymer solutions which on drying, by the evaporation of the solvent, produce ultrathin polymeric structures (Li & Xia, 2004). The electrospun nanostructures morphology and diameter are affected by the solution properties and by the process parameters and, for certain materials, reduced size capsules can be obtained when adjusting both. In this case, the electrospinning process is normally referred to as "electrospraying" due to the non-continuous nature of the structures obtained (Lopez-Rubio & Lagaron, 2012). Capsules and, thus, electrospraying, are generally preferred for food and nutraceutical applications, since apart from facilitating handling and subsequent incorporation into different products, they also present greater surface to volume ratio and, thus, are expected to have better release profiles than fibers (Hong, Li, Yin, Li, & Zou, 2008).

Folic acid is an essential micronutrient which cannot be synthesized by humans and, thus, it must be ingested through the diet (Lopera, Guzman, Cataño & Gallardo, 2009). Folic acid is the synthetic form of folates, a broad group of compounds with vitamin functionality (Bakhshi, Nangrejo, Stride & Edirisinghe, 2013). Specifically, folic acid is a water-soluble vitamin which is vital for a variety of physiological functions in humans. It plays an important role in the prevention of neural tube defects in infants and might decrease the likelihood of developing vascular diseases and some cancers (Liang, Zhang, Zhou & Subirade, 2013). According to these beneficial effects, the European

Regulations (Regulations 1924/2006; 1925/2006) allow the addition of folic acid in food and when its content provides more than 15% of the recommended daily amount, eight different health claims related to the beneficial effects of this vitamin are allowed in the food label (Regulation 432/2012). However, folic acid undergoes degradation reactions when it is exposed to light, temperature, moisture, acid or alkaline medium and oxygen atmosphere (Lopera et al., 2009). Therefore, the encapsulation of this bioactive ingredient is a plausible option to improve its stability and to assure its bioactivity within the food product during commercialization.

Regarding the matrix materials employed for encapsulation, food hydrocolloids are very convenient for nutraceutical and food applications, since many of them are soluble in aqueous solutions, thus, avoiding toxicity problems. Specifically, in the case of folic acid, there are some works which have shown the feasibility of spray drying for the encapsulation of this bioactive molecule within food hydrocolloids obtaining proper encapsulation efficiencies (Lopera et al., 2009). However, electrospinning from hydrocolloidal aqueous solutions has proven difficult due to several factors such as the polycationic nature or the low chain flexibility of these materials which complicates chain entanglements (essential for fiber formation) (Kriegel, Kit, McClements & Weiss, 2009). Moreover, the high surface tension of water, as well as, the ionization of water molecules at high voltages in an air environment, also complicates the electrospinning process. Therefore, synthetic polymers such as polyethylene oxide (PEO) (Alborzi, Lim & Kakuda, 2013) or multiple stages processes (Bakhshi et al., 2013) have been employed to date for the folic acid encapsulation through the electrospinning technology. Recent works have demonstrated that it is possible to obtain hydrocolloid-based encapsulation structures using electrospraying through the proper adjustment of

115 the aqueous solution properties (mainly surface tension and viscosity) upon addition of
116 several additives (surfactants and gums) (Pérez-Masiá, Lagaron & Lopez-Rubio, 2014a
117 and 2014b).

118 The aim of this work was to compare the more traditional spray drying technique (but
119 using a novel nanospray drying device able to obtain smaller encapsulation structures)
120 with the electrospraying methodology for folic acid encapsulation using two different
121 food hydrocolloid matrices (a whey protein concentrate and a commercial resistant
122 starch). Initially, whey protein concentrate (WPC) was used since it has excellent
123 functional characteristics, it is a low cost ingredient and it has proven useful for the
124 encapsulation of several functional ingredients through electrospinning (Lopez-Rubio &
125 Lagaron, 2011 and 2012). Additionally, a commercial resistant starch (derived from
126 corn starch) with trade name Fibersol was also employed for folic acid encapsulation, as
127 it was also the aim to compare the performance, in terms of protection, of a protein and
128 a carbohydrate matrix. Specifically, a resistant starch was used as it could provide an
129 additional beneficial prebiotic effect (Topping & Clifton, 2001). Both materials are
130 dispersable in aqueous solutions and, thus, they are very convenient for nutraceutical
131 applications. The encapsulation structures obtained through the two methodologies were
132 characterized, and a comparative evaluation of the encapsulation efficiency and folic
133 acid stability, under different storage conditions, and after applying a heating process
134 were ascertained.

135

136 **2. Materials and methods**

137 2.1. Materials

Whey protein concentrate (WPC) was kindly donated by ARLA (ARLA Food Ingredients, Viby, Denmark). Under the commercial name Lacprodan® DI-8090, the composition per 100 g of product consisted of ~80 g of protein, ~9 g of lactose, and ~8 g of lipids, being the rest water and minerals like sodium and potassium. The commercial resistant starch was Fibersol® (www.fibersol.com) commercial grade, manufactured by ADM/Matsutani (Iowa, USA). Guar Gum was purchased at Capers Community Markets (Vancouver, Canada). Folic acid (>97% purity) and the surfactant Span 20 were supplied by Sigma-Aldrich (Spain). All products were used as received without further purification.

2.2. Preparation of the solutions

The solutions were prepared depending on the encapsulation technology by dissolving 0.4 or 20% w/v of the matrix hydrocolloids in water for the spray drying or the electrospraying technique, respectively. The concentration of the matrices used in the solutions had been previously optimized, so as not to block the spraying head (in the case of spray drying) and to get the required chain entanglements for capsule formation (in the case of electrospraying). Additionally, 0.5wt.% of Guar gum with respect to the biopolymer matrix was incorporated, together with the hydrocolloid, in the resistant starch solutions. Span 20 was also added to the solutions to attain 5 wt.% with respect to the hydrocolloids weight. These solution compositions were selected as it had been previously optimized for these specific encapsulating matrices (Perez-Masiá et al., 2014b). When folic acid was incorporated, 1.5 wt.% of the bioactive with respect to the polymers weight was added. The solutions were stirred at room temperature until homogeneous dispersions of all the components were obtained.

2.3. Encapsulation through spray drying

The solutions with and without folic acid were spray-dried using a Nanospray-dryer B-90 (Büchi, Switzerland) with a 0.7 µm membrane cap. The solutions were introduced into the equipment through a silicone wire, which was connected to the spraying head of the equipment. The air flow was ~140 L/h with an inlet and outlet temperatures of 90ºC and 45ºC, respectively.

2.4. Encapsulation through electrospraying

The electrospinning apparatus, equipped with a variable high-voltage 0-30 kV power supply, was a Fluidnatek® LE-10 purchased from BioInicia S.L. (Valencia, Spain). Solutions with and without folic acid were introduced in a 5 mL plastic syringe and were electrospun under a steady flow-rate using a stainless-steel needle. The needle was connected through a PTFE wire to the syringe. The syringe was lying on a digitally controlled syringe pump while the needle was in horizontal towards a copper grid used as collector. The electrospraying conditions for obtaining the capsules were optimized and fixed at 0.15 mL/h of flow-rate, 10 kV of voltage and a tip-to-collector distance of 9-11 cm.

2.5. Characterization of the hydrocolloid/folic acid solutions

The apparent viscosity (η_a) of the hydrocolloid solutions was determined using a rotational viscosity meter Visco Basic Plus L from Fungilab S.A. (San Feliu de

Llobregat, Spain) using a Low Viscosity Adapter (LCP). The surface tension of the biopolymer solutions was measured using the Wilhemy plate method in an EasyDyne K20 tensiometer (Krüss GmbH, Hamburg, Germany). The conductivity of the solutions was measured using a conductivity meter XS Con6 (Labbox, Barcelona, Spain). All measurements were made at 25ºC.

2.6. Optical microscopy

Optical microscopy images were taken using a digital microscopy system (Nikon Eclipse 90i) fitted with a 12 V, 100W halogen lamp and equipped with a digital imaging head which integrates an epifluorescence illuminator. A digital camera head (Nikon DS-5Mc) with a 5 megapixel CCD cooled with a Peltier mechanism was attached to the microscope. Nis Elements software (Nikon Instruments Inc., USA) was used for image capturing.

2.7. Scanning Electron Microscopy (SEM)

The morphology of the encapsulation structures was examined using SEM on a Hitachi microscope (Hitachi S-4100) after having been sputtered with a gold-palladium mixture under vacuum. All SEM experiments were carried out at 10 kV. Capsule diameters were measured by means of the Adobe Photoshop CS3 software from the SEM micrographs in their original magnification.

2.8. Attenuated total reflectance infrared spectroscopy (ATR-FTIR)

206 Attenuated total reflectance infrared spectroscopy (ATR-FTIR) was used to analyze the
207 molecular organization of the capsules. The experiments were recorded in a controlled
208 chamber at 21ºC and 40% RH coupling the ATR accessory GoldenGate of Specac Ltd.
209 (Orpington, UK) to a Bruker (Rheinstetten, Germany) FTIR Tensor 37 equipment. The
210 samples obtained using both encapsulation techniques were placed onto the ATR crystal
211 and all the spectra were collected within the wavenumber range of 4000–600 cm^{-1} by
212 averaging 15 scans at 4 cm^{-1} resolution. Analysis of the spectral data was performed by
213 using Grams/AI 7.02 (Galactic Industries, Salem, NH, USA) software.

214

215 2.9. Folic acid encapsulation efficiency and stability assay

216 To assess the encapsulation yield, the amount of folic acid was determined by HPLC
217 following the methodology described by Konings (1999) using a Merck-Hitachi 7000
218 (Merck, Darmstadt, Germany) HPLC equipped with an ultraviolet detector (LaChrom,
219 Merck-Hitachi, model 7400), a LiChrosphere® 100 RP-18 (5 µm) column (Merck,
220 Darmstadt, Germany) protected with a guard column (LiChroCART® 4-4, Merck,
221 Darmstadt, Germany). The column was first eluted with a gradient of acetonitrile and 30
222 mmol/L phosphate buffer (potassium phosphate and ortho-phosphoric acid 85%, pH
223 2.2) at a flow rate of 0.9 mL/min. The gradient started at 6% acetonitrile, which was
224 maintained isocritically for the first 6 min, and then the acetonitrile concentration was
225 increased to 25% over 24 min and decreased back to 6% after 5 min. The injection
226 volume was 40 µL. The running time was 40 min, and the time between injections was
227 20 min. Peak identification and quantification was based on the retention time compared
228 with non-encapsulated folic acid measuring the UV absorbance at 290 nm. All samples

were analysed in quadruplicate and data were expressed as µg/ml and the losses percentage of folic acid along the storage and heating were also calculated.

The encapsulation efficiency (EE) was calculated using the following equation:

$$EE\ (\%) = \frac{\text{Folic acid in the capsules (µg/mg of matrix) x 100}}{\text{Folic acid added to the solutions (µg/mg of matrix)}} \quad \text{(Eq. 1)}$$

The stability of encapsulated folic acid was carried out taking into consideration three different situations that can be found in the food industry: dissolution in aqueous media, storage in dryness and temperature exposure. It is important to note that the heat treatment was applied after storage in dryness during 2 months. Aqueous solutions were prepared weighing 1 mg of capsules and dissolving them in 1 mL of deionised water. Immediately, the amount of folic acid was determined by HPLC, as explained above, and the solutions were stored at room conditions (23ºC and 65% relative humidity). Samples were tightly closed to avoid water losses though evaporation, and folic acid content was analysed every 15 days (up to 60 days). In addition, the folic acid capsules were stored in dryness in the same room conditions (temperature, humidity), in the presence and absence of light and, in a similar way, the content of this bioactive was analysed every 15 days (up to 60 days). To evaluate the stability when exposed to high temperature, the samples stored during 2 months were placed in a heating oven at 120ºC during 20 minutes. Every 5 minutes during the heating process, samples were taken out of the oven and re-suspended in distilled water (1 mg/mL w/v) before being injected in the HPLC to determine the amount of folic acid. In parallel, a solution of 1 mg/ml of pure folic acid was assayed following exactly the same methodology described above, to evaluate the effect of encapsulation process in the stability of this bioactive.

The loss percentages were calculated with the following equation:

$$\text{Loss (\%)} = \frac{\text{Initial folic acid content} - \text{folic acid content at time t}}{\text{Initial folic acid content}} \times 100 \qquad \text{(Eq. 2)}$$

2.10. Statistical analysis

A statistical analysis of data was performed through analysis of variance (ANOVA) using Statgraphics Plus for Windows 5.1 (Manugistics Corp., Rockville, MD). Homogeneous sample groups were obtained by using LSD test (95% significant level). In the stability study ANOVA was applied taking into consideration the sample type and storage time, and a post-hoc test was used to establish the differences among mean values at significant level of 95%.

3. Results and discussion

3.1. Solution properties

Before encapsulation, optimum solution parameters were set for both technologies. In the case of spray drying, much diluted solutions were produced in order to avoid the spraying head membrane blockage. Therefore, very low apparent viscosity values were obtained (< 2cP; data not shown).

In the case of electrospraying, the successful development of encapsulation structures using this technology strongly depends on the solution properties and, hence, an initial optimization of solution composition was carried out. From a screening study, it was

277 seen that resistant starch solutions led to very low apparent viscosity values, which
278 resulted in unstable jetting and no structures were formed from these solutions.
279 Therefore, a thickening agent, specifically guar gum, was added to this solution in order
280 to increase the viscosity and facilitate molecular entanglements between the
281 carbohydrate chains. Moreover, guar gum also helped to avoid folic acid precipitation in
282 the syringe during the electrospraying encapsulation process. It was also observed that,
283 even though high protein and carbohydrate concentrations were used, the surface
284 tension values of the aqueous solutions were still too high to allow capsule's formation.
285 Hence, the surfactant Span-20 was incorporated in the solutions, since it has been
286 previously seen that this compound effectively reduces solution surface tension,
287 favouring the electrospraying process and, thus, capsules formation (Pérez-Masiá et al.,
288 2014a and 2014b). The gum and the surfactant were also included in the spray-drying
289 solutions in order to obtain the same shell materials in both cases. Table 1 shows the
290 apparent viscosity, the surface tension and the electrical conductivity of the final
291 hydrocolloid-based electrospraying solutions with and without folic acid. It can be
292 observed that low apparent viscosity values were obtained for both biopolymeric
293 solutions in the absence of folic acid. This fact was mainly due to the low molecular
294 weight of both hydrocolloids and, also to the concentration of these biopolymers in the
295 solutions. This parameter was kept low in order to limit the chain entanglements of the
296 biopolymers and, thus, obtain discontinuous particles instead of continuous fibers. In
297 the presence of folic acid it was observed that the apparent viscosity of the resistant
298 starch solution was not significantly modified. In contrast, the WPC solution presented
299 an enhanced apparent viscosity suggesting that the folic acid was interacting with the
300 protein. With regard to the surface tension, it was seen that the incorporation of the
301 surfactant (Span 20) in all the solutions decreased this parameter to suitable values for

302 stable electrospraying (Pérez-Masiá et al., 2014a and 2014b). From Table 1 it can also
303 be observed that the electrical conductivity of the WPC solutions was much higher than
304 that of the resistant starch ones, due to the ionic nature of the protein in solution.

305 INSERT TABLE 1 ABOUT HERE

306

307 3.2. Morphology of the capsules

308 Initially, optical microscopy using a fluorescence source was used to confirm capsules
309 formation, as well as to confirm the proper encapsulation of folic acid, since this
310 vitamin presents fluorescence in the UV range, while the matrix materials did not
311 presented this feature. Figure 1 shows the optical micrographs of the hydrocolloids/folic
312 acid capsules developed and it was observed that both matrices led to the formation of
313 spherical capsules, although some agglomerations were seen in the starch-based
314 materials. Moreover, from the fluorescence images it was also seen that folic acid was
315 properly encapsulated in both matrices regardless the encapsulation technique
316 employed.

317 INSERT FIGURE 1 ABOUT HERE

318

319 The capsules obtained were also observed through scanning electron microscopy (SEM)
320 in order to better characterize their size and morphology. Figure 2 shows the SEM
321 images and the corresponding size distribution of the resulting capsules attained through
322 nanospray-drying and electrospraying. It was observed that spherical submicron and
323 micron capsules were obtained through both techniques, although spray-drying

generally led to bigger average diameters and broader size distribution of the capsules, since it is more difficult to control the morphology and size of the particles with this technology. On the contrary, electrospraying led to an enhanced control over the size of the capsules and generally, narrower size distributions were found when using this technique. Moreover, smaller structures were attained when using this technique, which could favour the incorporation and dispersion of the capsules within food products without affecting their textural characteristics. However, it was seen that when resistant starch was used as encapsulating matrix, some big structures were obtained even through electrospraying, which could be due to the presence of guar gum in the solutions. A previous study showed that gums retained water causing an incomplete drying of the electrospraying jet and leading to more unstable electrospraying process, fact that could result in more heterogeneous capsule sizes (Pérez-Masiá et al., 2014b). Nevertheless, it was observed that the incorporation of guar gum was essential, not only for capsule formation, but also in order to avoid a premature folic acid precipitation during the experiments, keeping the folic acid in suspension with resistant starch within the plastic syringe. It was also seen that the incorporation of the folic acid did not considerably affect the morphology of the capsules.

INSERT FIGURE 2 ABOUT HERE

3.3. Changes in molecular organization

Attenuated total reflectance infrared spectroscopy (ATR-FTIR) experiments were carried out in order to identify molecular changes in the hydrocolloids due to the encapsulation processes, as well as to detect interactions between the hydrocolloid

347 matrices and the folic acid. The FTIR spectra of the capsules' individual components
348 have been provided as supplementary data (*Supplementary Figure 1S*). It was observed
349 that folic acid was mainly characterized by the O-H (~3545 cm^{-1}), N-H (~3418 and
350 ~3323 cm^{-1}) and the C=O stretching vibrations (~1695 cm^{-1}), the bending mode of the
351 N-H group (~1606 cm^{-1}) and the absorption of the phenyl ring (~1485 cm^{-1}) (Hammud
352 et al., 2013; Bakhshi et al., 2012). The IR spectrum from WPC is mainly characterized
353 by the amide I and II bands at around 1633 and 1516 cm^{-1}, respectively (Xiaozhan et al.,
354 2009). Regarding the IR spectrum of the resistant starch, it presented the characteristic
355 carbohydrate overlapped bands from 800 to 1200 cm^{-1} related to the stretching
356 vibrations of C-O and C-C groups, and the bending vibration of C-O-H group (Wolkers,
357 Oliver, Tablin, & Crowe, 2004; Kacurakova & Mathlouthi, 1996). In this region, a
358 characteristic band from resistant starch which was not overlapped with the vibrational
359 bands from folic acid was seen at around 1008 cm^{-1}, although guar gum also presented a
360 similar band nearby this wavenumber. Therefore, the amide bands of the WPC and the
361 starch band at 1008 cm^{-1} were studied to analyse the effect of the encapsulation
362 techniques and the incorporation of folic acid on the molecular organization of the
363 capsules.

364 Figure 3 displays the selected characteristic bands of the unprocessed encapsulating
365 matrices and those from the capsules with and without folic acid. It was seen that,
366 probably due to the low concentration of the bioactive within the capsules, the folic acid
367 signal was not apparent in the infrared spectra. Nevertheless, the presence of the vitamin
368 produced some variations in the spectra of the capsules. From Figures 3A and 3B it was
369 seen that amide I and II bands were shifted towards higher wavenumbers in the spectra
370 from the capsules, suggesting that some molecular changes occurred in the whey protein

as a consequence of capsule formation using both technologies. Specifically, the spray drying technique led to a greater displacement of the spectral bands than the electrospraying method probably because the former used high temperatures that could affect more strongly the molecular bonding of the WPC chains. It was also seen that the capsules which contained the folic acid presented narrower bands than those without the bioactive, which suggested that the incorporation of folic acid led to a greater molecular order. The incorporation of the vitamin also produced a greater displacement of the amide II band when compared to those without the bioactive, suggesting some kind of interaction between the proteins and the folic acid (as also inferred by the previously described increase in solution apparent viscosity). In fact, it has been already reported that the main proteins found in whey obtained from bovine milk have the ability to interact with folic acid through different mechanisms (Liang et al., 2013). It has also been seen that folic acid is able to conjugate to different polymers via an amide linkage through the carboxylic group of the bioactive, which is evident at around 1695 cm^{-1} (Sudimack & Lee, 2000; Teng, Luo, Wang, Zhang & Wang, 2013). Thus, the shifts toward higher wavenumber of the amide II band could be ascribed to the interaction between the protein matrix and the folic acid. Regarding the resistant starch infrared spectra, it was observed that the band at 1008 cm^{-1} located in the characteristic carbohydrate region, was also shifted towards higher wavenumbers when the starch was processed through both encapsulation techniques (Figures 3C and 3D). However, in this case, the shift could also be due to the presence of guar gum, which presented a band nearby this wavenumber. Nevertheless, it is worth noting that the band displacement was greater for the spray dried structures. Again, this result could be explained by the higher temperature applied during this encapsulation process, as well as to a greater amount of guar gum in the spray dried capsules due to a better incorporation of capsules

components through this technique. The differences in incorporation of the various compounds in the capsules could be explained by the different processing conditions during encapsulation, i.e., while during the nanospray drying process the solutions were continuously stirred, during electrospraying the solutions were left static within the syringes and, thus, partial precipitation of some compounds took place during the electrospraying process. From Figures 3C and 3D it was also observed that in this case the incorporation of the folic acid also produced narrower bands, suggesting a greater molecular order of the structures. Nevertheless, the incorporation of the bioactive did not considerably affect the molecular organization of the resistant starch capsules. Thus, it could be concluded that these materials did not interact with each other, but the folic acid was physically confined within the starch capsules.

INSERT FIGURE 3 ABOUT HERE

3.4. Encapsulation efficiency and folic acid stability

HPLC chromatograms from encapsulated and non-encapsulated folic acid and from the encapsulation matrices at the beginning of the stability study and immediately after they were dissolved in water (0 days) can be found in the supplementary material (*Supplementary Figure 2S*). Although both the encapsulation matrices and the folic acid absorb at 290 nm, it was observed that the peaks were effectively separated in the chromatograms, with an elution time around 27-30 minutes for folic acid and 35 minutes for the matrices. This separation could probably be achieved by the buffer with a pH 2.2, since at this pH a decrease on the interactions between folic acid and the different encapsulation matrices has been previously reported (Liang & Subirade, 2012).

419 The peak area of the folic acid also showed differences between samples, indicating that
420 the different biopolymers and encapsulating technologies could induce variability of
421 results in the amount of encapsulated folic acid.

422 Table 2 shows the amount of folic acid in the different encapsulation structures as a
423 function of storage time under different conditions (after dissolving the capsules in
424 water and the stored dry capsules in the presence or absence of light). Taking into
425 consideration that the initial amount of folic acid added to the different solutions was
426 1.5% (15 μg/mg), from the initial data, it was seen that the encapsulation efficiency was
427 greater for the WPC matrix than for the resistant starch. This result was probably related
428 to the interactions taking place between the protein matrix and the bioactive, which
429 would facilitate the incorporation of the folic acid within the protein capsules. From
430 these data, it was also observed that there were not significant differences in
431 encapsulation efficiency when using nanospray drying or electrospraying. When
432 comparing the encapsulation yield with other works, it was seen that similar loadings
433 were found when using food hydrocolloids as encapsulation matrices (Madziva,
434 Kailasapathy & Phillips, 2004).

435 INSERT TABLE 2 ABOUT HERE

436 Regarding the stability of folic acid within the capsules, a different behaviour was
437 observed depending on the storage conditions, i.e. aqueous solution or dryness. Figure 4
438 shows the stability of folic acid, expressed as percentage of the initial amount of
439 bioactive found in the capsules, in the different structures assayed during 60 days for
440 capsules in aqueous solutions (Figure 4A) and for capsules in dry conditions under
441 natural light (Figure 4B) and in darkness (Figure 4C). It was seen that for capsules in
442 aqueous solution, the amount of folic acid significantly decreased after 15 days in all

443 cases except in the WPC capsules obtained through nanospray drying. In these samples,
444 the losses of the synthetic folate were around 60%, remaining quite stable along time
445 until 45 days. On the other hand, the folic acid degradation was extremely marked in the
446 resistant starch capsules, in which losses were higher than 90% of the initial amount.
447 This result can be explained by the high solubility of this polysaccharide in water,
448 which led to a very quick release of the bioactive which was, thus, no longer protected.
449 From Figure 4A it was also observed that the capsules obtained through spray drying
450 displayed better protection ability than those obtained through electrospraying. As it
451 was seen from ATR-FTIR results, the two encapsulation techniques led to different
452 matrix conformations and, from the results it appeared that spray drying could provide
453 more compact structures which would consequently had improved moisture resistance.
454 Nevertheless, it is worth noting that non-encapsulated folic acid drastically reduced its
455 content, only remaining 1% after 15 days of storage and, hence, even the highly soluble
456 resistant starch capsules provided improved folic acid stability.

457 In contrast with the slight improvements observed when the storage experiments were
458 carried out with the capsules immersed in aqueous solutions, from Figures 4B and 4C it
459 was observed that under dry conditions, encapsulation effectively provided a great folic
460 acid stability during storage time, especially when the encapsulating matrix was WPC
461 independently of the encapsulation technology. The WPC capsules were able to almost
462 keep the bioactive stability at 100% in darkness conditions, while 40% of non-
463 encapsulated folic acid was degraded. From these figures it can also be seen that
464 resistant starch capsules did not provide a great protective effect when compared with
465 non-encapsulated folic acid. However, it should be noted, that the relative humidity of
466 the storage room in dry conditions was around 65% and, thus, starch capsules could be

at least partially dissolved, leaving the folic acid exposed to the ambient conditions. It is interesting to note that, upon light exposure (cf. Figure 4B), faster degradation kinetics were observed for the folic acid present in the resistant starch capsules than for the non-encapsulated bioactive. It is hypothesized that this result was related to the processing and storage conditions, i.e. the as-received dry bioactive could remain more stable at ambient conditions when exposed to light than the folic acid molecules that had been dispersed in solution for capsule formation. Although the solvent was evaporated during encapsulation using both techniques, the resistant starch matrix, as previously mentioned, was highly hydrophilic and, thus, at ambient conditions it would adsorb water, thus promoting folic acid molecular mobility and degradation upon light exposure.

INSERT FIGURE 4 ABOUT HERE

Regarding the folic acid stability upon high temperature exposure, it is important to note that this study was carried out after the stability assay in dryness conditions, and thus, the capsules were not freshly prepared, but they were studied after 2 months of storage. Figure 4D shows the stability of the different samples after thermal treatment at 120ºC for different times.

It was seen that non-encapsulated folic acid was completely degraded after only 5 minutes at 120ºC, as no peaks were detected through HPLC. In contrast, the encapsulation structures were able to keep part of the bioactive in good conditions during the experiment. In general, it was observed that the percentage of losses of folic acid were directly proportional to the time of treatment, providing higher stability WPC than resistant starch capsules. This fact could be due to the interaction between folic acid and whey proteins, since it has been previously reported that this interaction could

act as a protective factor. Specifically, Liang & Subirade (2012) observed that this biopolymer induce a thermal stabilization of synthetic folate until 85ºC. Nevertheless, it is worth noting that this study was done with stored samples, rather than fresh capsules as the rest of the stability studies. Therefore, folic acid could be already partially degraded, and further studies should be carried out in order to better assess the protective effect of capsules during heating conditions.

4. Conclusions

In this work, folic acid was encapsulated using two different matrices (WPC and a commercial resistant starch) and two different encapsulation techniques (spray drying and electrospraying). This study showed that electrospraying can be used as a promising technology in the food industry for encapsulation applications, since the capsules obtained presented similar morphological characteristics than those obtained through spray drying. Furthermore, it does not require heating or the use of organic agents which could destroy some sensitive encapsulated nutrients or cause toxicity problems. Specifically, results showed that spherical submicron and micron capsules were obtained for all the compositions assayed, although electrospraying generally allowed an enhanced control over the size distribution of the capsules and smaller capsules. Concerning the encapsulation efficiency, it was observed that there were not significant differences between both encapsulation technologies. However, WPC led to higher encapsulation yields than resistant starch, probably because of the interaction between the protein matrix and the folic acid which facilitated the incorporation of the bioactive within the capsules. With regard to the biopolymers used, it was observed that WPC protected the folic acid against the degradation during storage in both situations

(aqueous solution and dryness). On the contrary, resistant starch capsules provided lower stability to folic acid when capsules were suspended in aqueous solution. Nevertheless, protection was greater in dryness conditions; hence these capsules might be better applied in dry foods. Regarding the folic acid stability under thermal exposure, even though promising results were obtained, further studies should be carried out in order to determine the potential applications.

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FIGURE CAPTIONS

Figure 1. Optical micrographs (under normal illumination and using a fluorescence source to distinguish encapsulated folic acid) of WPC/Folic acid (A,B) and Resistant Starch/Folic acid (C,D) capsules obtained through nanospray drying (A, C) and electrospraying (B, D). Scale bar corresponds to 10 μm.

Figure 2. Selected SEM images and size distribution of WPC, WPC/folic acid, resistant starch and resistant starch /folic acid capsules developed through nanospray drying (A, B, C and D, respectively. Scale bars correspond to 5 μm) and electrospraying (E, F, G and H, respectively. Scale bars correspond to 2 μm).

Figure 3. ATR-FTIR spectra of folic acid (grey lines), neat hydrocolloids (black lines), capsules without folic acid (dashed lines) and of the capsules with folic acid (dotted lines) for the WPC nanospray dried (A) and electrosprayed materials (B) and the resistant starch nanospray dried (C) and electrosprayed (D) materials.

Figure 4. Stability of folic acid, expressed as percentage of the initial amount, in the different capsules assayed along storage: non encapsulated folic acid (cross); WPC/Folic acid capsules obtained through nanospray drying (rhombus) and electrospraying (square); and Resistant starch/folic acid capsules obtained through nanospray drying (triangle) and electrospraying (circle). Figure A represents the aqueous solutions of encapsulated folic acid, and Figures B and C represent the stability of folic acid in the capsules stored in dry conditions both under natural light and in darkness, respectively. Figure D represents the stability of folic acid along the thermal assay during 20min.

Figure 1

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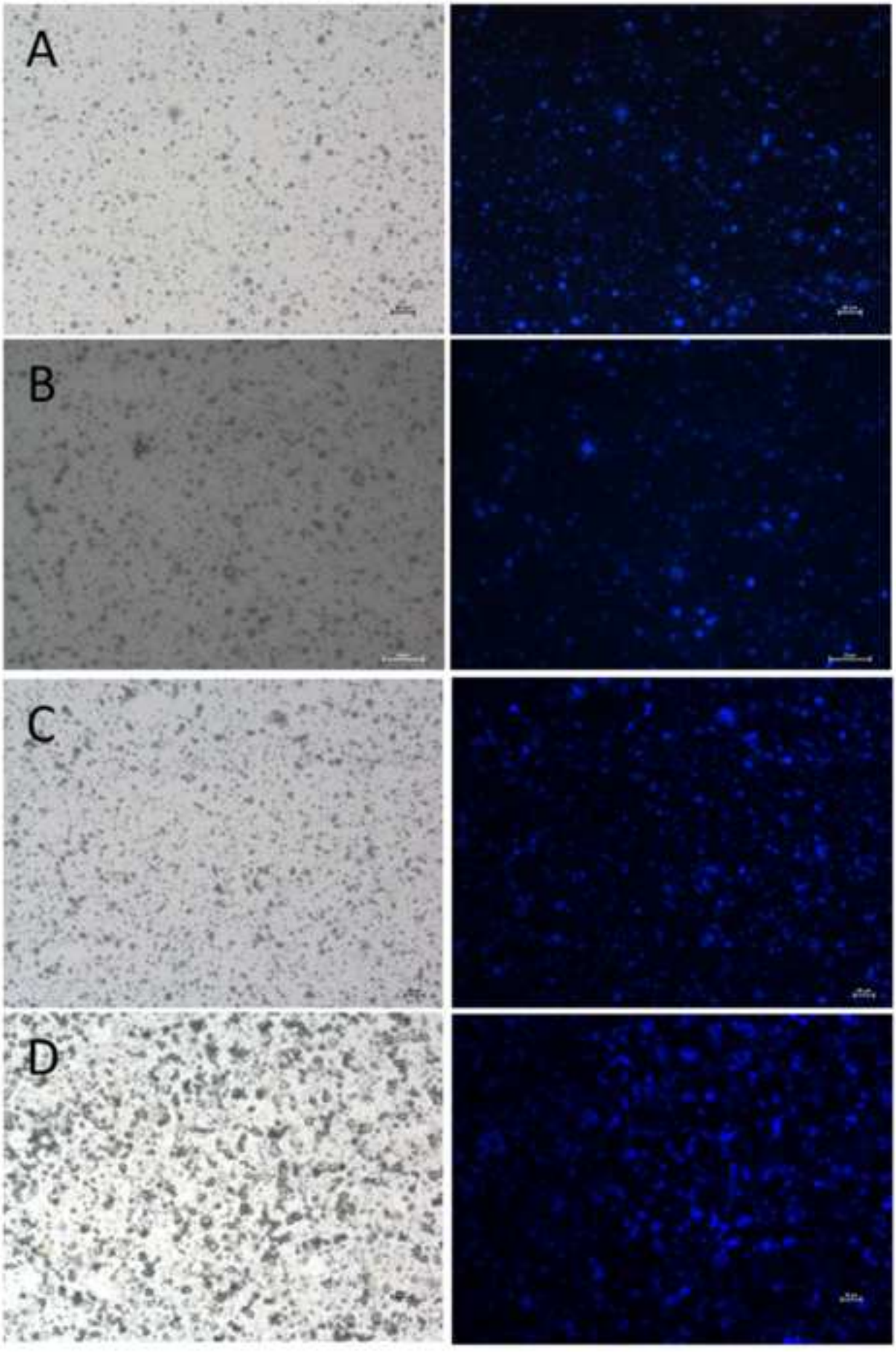

Figure 2

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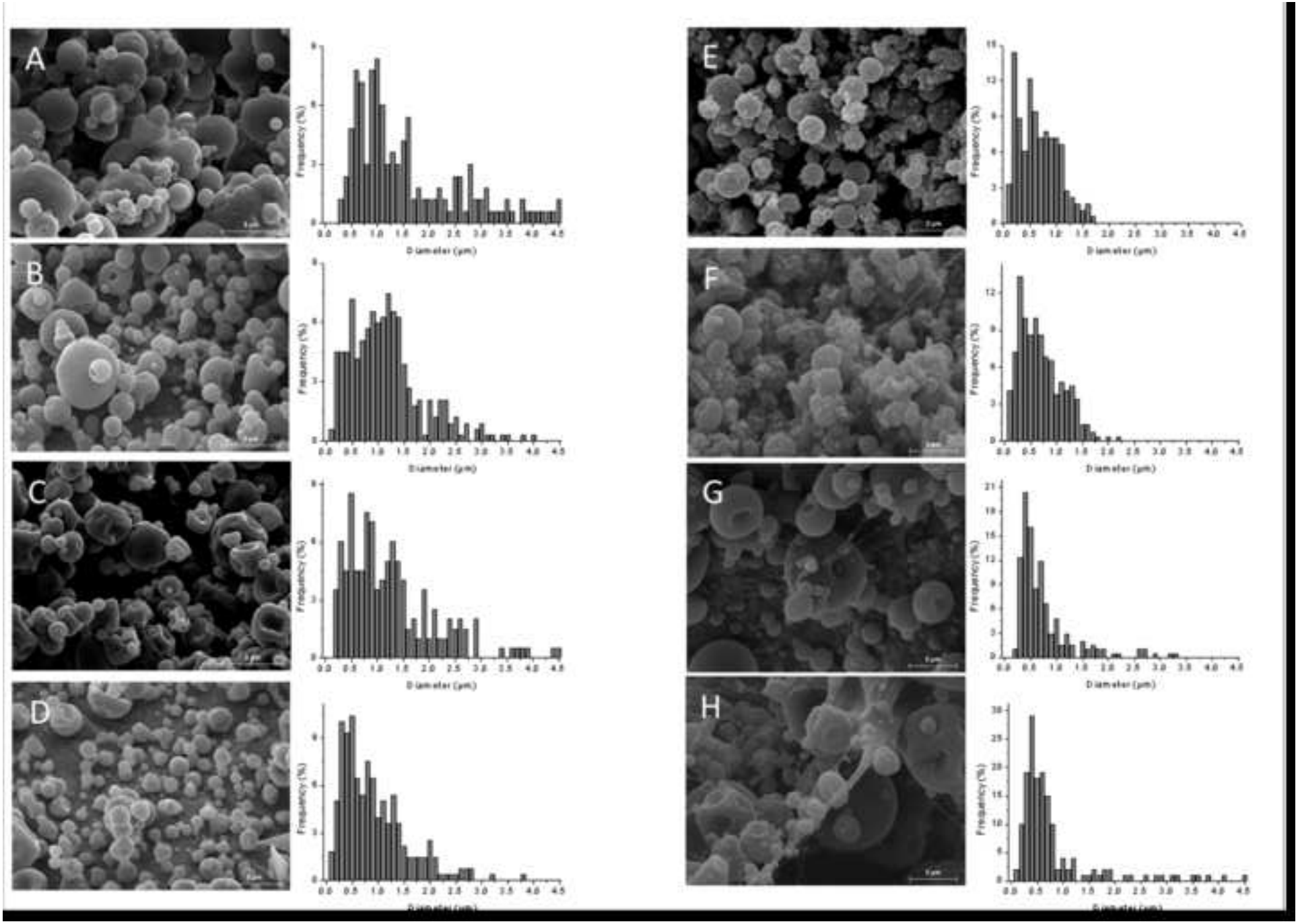

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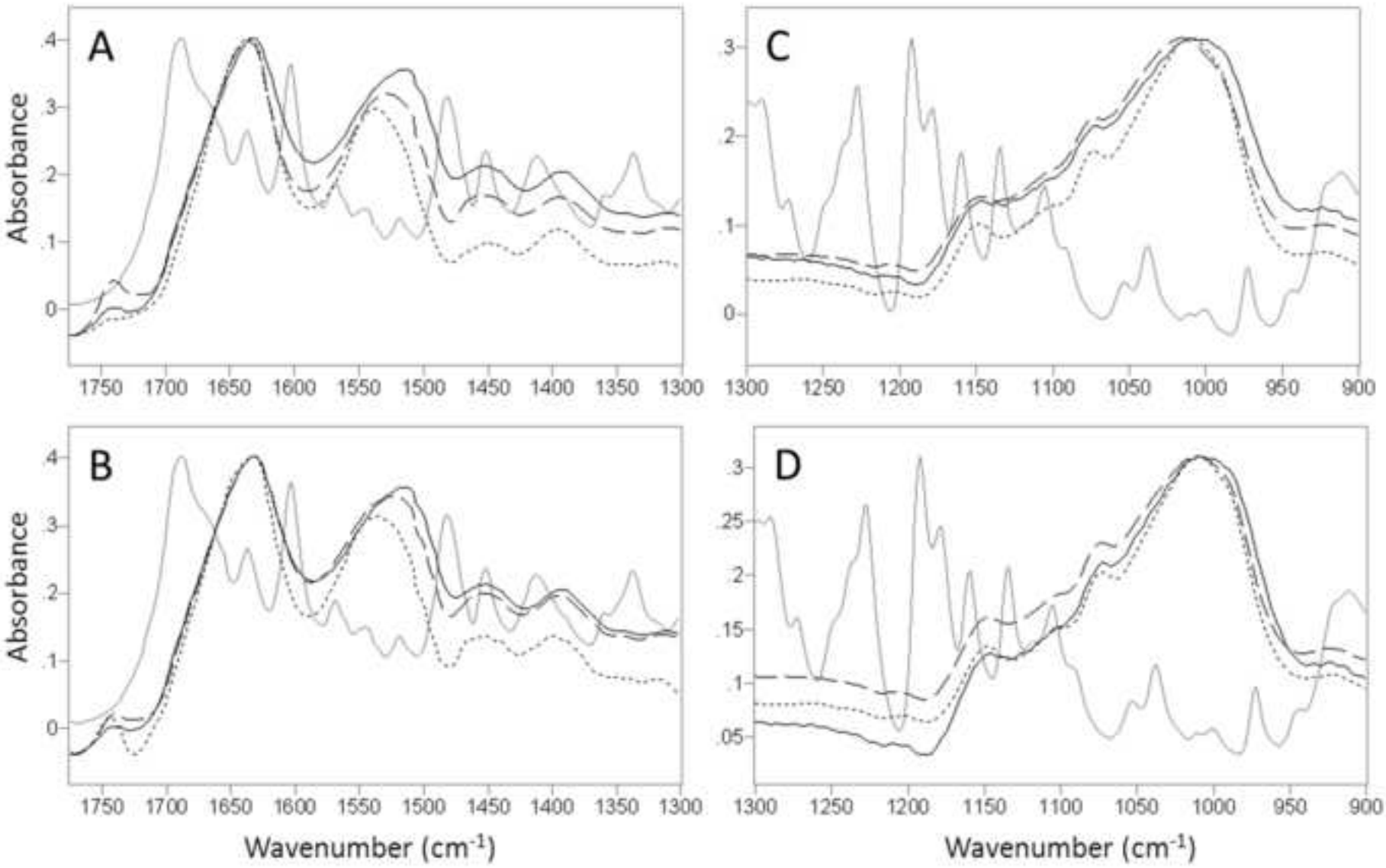

Figure 4

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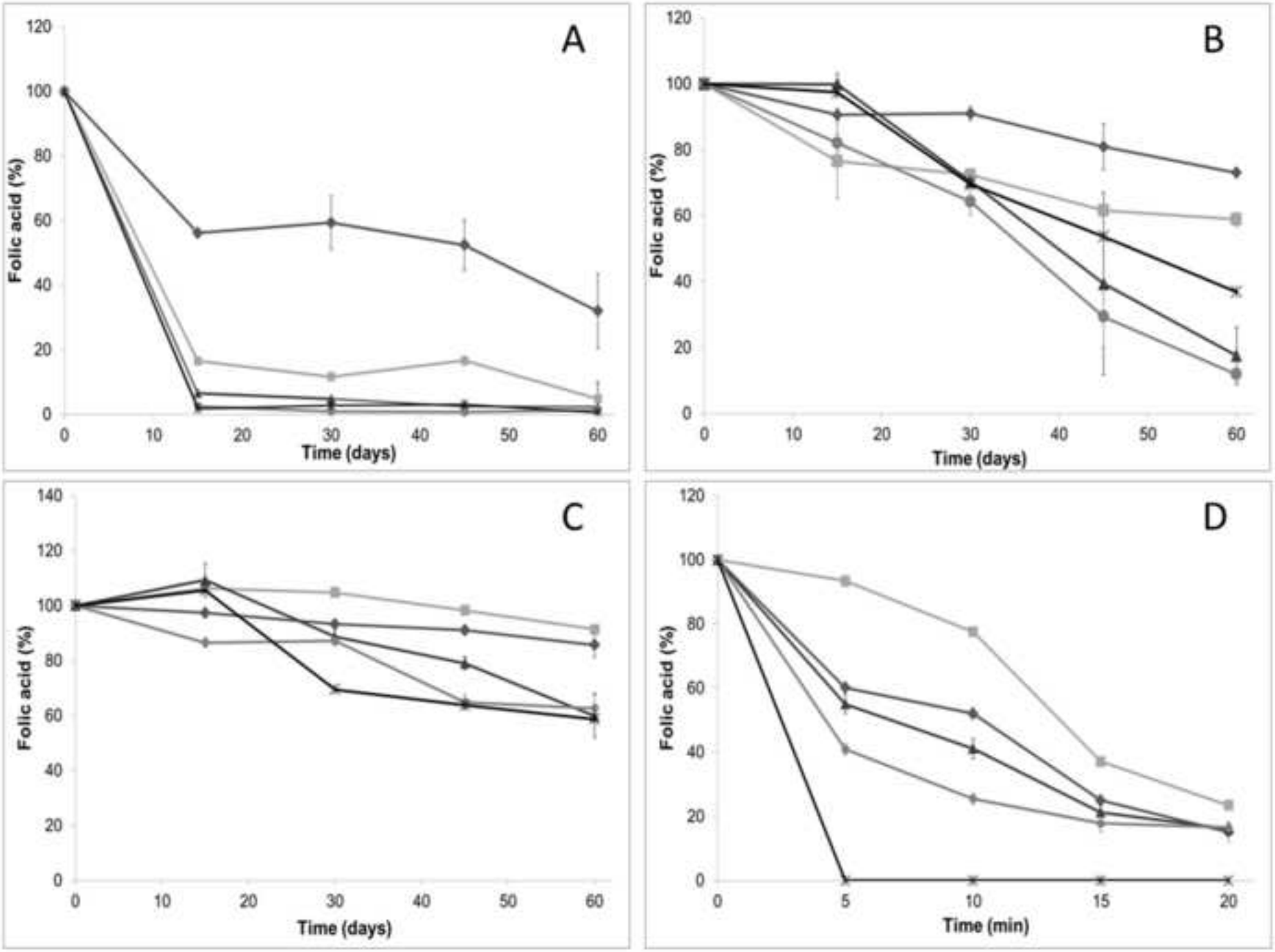

Table 1

Table 1. Solution properties of the electrospraying solutions with the following compositions: WPC (20% w/v of WPC in water + 5 wt.% Span20); Resistant starch (20% w/v resistant starch in water + 0.5 wt.% guar gum + 5 wt.% Span20). The solutions with folic acid additionally contained 1.5 wt.% of the bioactive with respect to the biopolymer weight.

	Viscosity (cP)	Surface tension (mN/m)	Conductivity (μS)
WPC	5.56 ± 0.4^{a}	31.74 ± 0.7^{a}	1750 ± 12.1^{a}
WPC/Folic acid	198.11 ± 0.4^{b}	29.50 ± 0.3^{b}	1690.67 ± 6.7^{b}
Resistant starch	5.52 ± 0.5^{a}	25.16 ± 0.8^{c}	37.25 ± 0.5^{c}
Resistant starch/Folic acid	5.86 ± 0.1^{a}	25.83 ± 0.2^{c}	39.37 ± 0.2^{d}

$^{a-d}$ Different letters in the same column show statistical significant differences ($p<0.05$).

Table 2

Table 2. Encapsulation efficiency and amounts of folic acid expressed as µg/mg of matrix during storage in water solution and in dry conditions under light and darkness during 60 days[1]. [1]Values are expressed as mean ± standard deviation.

	Encapsulation efficiency (%)	0 days	15 days	30 days	45 days	60 days
			Water solution			
WPC/Folic acid (spray drying)	83.9 ± 7.8^{a}	12.59 ± 0.83^{a}	7.07 ±0.14^{a*}	7.47 ± 1.09^{a*†}	6.60 ± 1.03^{a*†}	4.03 ± 1.91^{a†}
WPC/Folic Acid (electrospraying)	80.8 ± 12.9^{a}	12.12 ± 1.37^{a}	2.00 ± 0.11^{b*}	1.41 ± 0.14^{b*}	2.02 ± 0.18^{b*}	0.59 ± 0.03^{b†}
Resistant starch/Folic acid (spray drying)	52.5 ± 7.6^{b}	7.87 ± 0.81^{b}	0.51 ± 0.03^{c*}	0.38 ± 0.09^{c*†}	0.20 ± 0.14^{c†}	0.18 ± 0.17^{c†}
Resistant starch/Folic acid (electrospraying)	44.0 ± 5.5^{b}	6.75 ± 0.43^{b}	0.17 ± 0.07^{d*}	0.06 ± 0.03^{d*†}	0.05 ± 0.01^{d†}	0.09 ± 0.05^{d*†}
			Dry/Natural light			
WPC/Folic acid (spray drying)	83.9 ± 7.8^{a}	12.59 ± 0.83^{a}	11.40 ± 0.24^{a*}	11.46 ± 0.26^{a*}	10.19 ± 0.87^{a*†}	9.19 ± 0.16^{a†}
WPC/Folic Acid (electrospraying)	80.8 ± 12.9^{a}	12.12 ± 1.37^{a}	9.27 ± 1.73^{b*}	8.77 ± 0.14^{b*}	7.46 ± 0.17 ^{b†}	7.13 ± 0.25^{b†}
Resistant starch/Folic acid (spray drying)	52.5 ± 7.6^{b}	7.87 ± 0.81^{b}	7.86 ± 0.26^{c*}	5.54 ± 0.05^{c†}	3.10 ± 2.17$^{c\dagger **}$	1.38 ± 0.68^{c**}
Resistant starch/Folic acid (electrospraying)	44.0 ± 5.5^{b}	6.75 ± 0.43^{b}	5.54 ± 0.19^{d*}	4.34 ± 0.28^{d†}	1.98 ± 0.63^{c**}	0.80 ± 0.11^{d***}
			Dry/Darkness			
WPC/Folic acid (spray drying)	83.9 ± 7.8^{a}	12.59 ± 0.83^{a}	12.26 ± 0.13^{a*}	11.74 ± 0.23^{a*}	11.47 ± 0.22^{a*}	10.79 ± 0.54^{a*}
WPC/Folic Acid (electrospraying)	80.8 ± 12.9^{a}	12.12 ± 1.37^{a}	12.88 ± 0.22^{a*}	12.70 ± 0.16^{a*}	11.92 ± 0.10^{a†}	11.08 ± 0.15^{a**}
Resistant starch/Folic acid (spray drying)	52.5 ± 7.6^{b}	7.87 ± 0.81^{b}	8.60 ± 0.47^{a*}	6.98 ± 0.26^{b†}	6.22 ± 0.18^{b†}	4.71 ± 0.62^{b**}
Resistant starch/Folic acid (electrospraying)	44.0 ± 5.5^{b}	6.75 ± 0.43^{b}	5.84 ± 0.12^{c*}	5.88 ± 0.11^{c*}	4.38 ± 0.18^{c†}	4.23 ± 0.37^{b†}

$^{a-d}$ Different letters in the same column for each storage condition (water solution, dry/natural light or dry/darkness) show statistical significant differences ($p<0.05$).

$^{*, \dagger}$ Different symbols in the same row show statistical significant differences ($p<0.05$) along storage time.

Supplementary Material

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